

AD_____

Award Number: W81XWH-04-C-0071

TITLE: Neuroimmune Effects of Inhaling Low Dose Sarin

PRINCIPAL INVESTIGATOR: Mohan L. Soporì, Ph.D.

CONTRACTING ORGANIZATION: Lovelace Biomedical and Environmental
Research Institute
Albuquerque, NM 87108

REPORT DATE: February 2008

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				<i>Form Approved</i> OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE 14-02-2008		2. REPORT TYPE Final		3. DATES COVERED 15 JAN 2004 - 14 JAN 2008	
4. TITLE AND SUBTITLE Neuroimmune Effects of Inhaling Low Dose Sarin				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-04-C-0071	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Mohan L. Sopori, Ph.D. Email: msopori@lrri.org				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Lovelace Biomedical and Environmental Research Institute Albuquerque, NM 87108				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The nerve gas sarin is a potent irreversible inhibitor of acetylcholine esterase that, in high doses, causes neurotoxicity, seizures, lung inflammation, and death primarily from respiratory failure. Even in subclinical doses, sarin suppresses the immune system and decreases serum corticosterone (CORT) levels. However, the mechanism and duration these effects are not known. Our results that sarin upregulates the mRNA expression of proinflammatory cytokines in the lung which is associated with the activation of MAP kinases (ERK1/2) and the transcription factor, NFkB. Sarin induced suppression of serum CORT level appear to be through its effects on the HPA axis. In addition, higher doses of sarin that causes respiratory failure in animals result primarily from functional loss of central respiratory chemoreceptors. Moreover, higher doses of sarin specifically damage the hippocampus and some regions of the cortex, and might explain the long-term neurobehavioral problems seen in the survivors of Japanese sarin terrorism. Taken together, these results suggest that, low doses of sarin affect adaptive and innate immune responses and depress CORT and ACTH levels, higher doses of sarin may cause death through loss of central respiratory chemoreceptors. Our preliminary results also suggest that exposure to high-doses of sarin (~LD50) may impair cognitive functions through injury to hippocampal and cortical neurons.					
15. SUBJECT TERMS Sarin; Neuroimmune Interactions; Biomarkers; Lung inflammation; Ventilatory Responses					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 14	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Table of Contents

ITEM	Page
Face Page	1
Abstract	1
Table of Contents	2
Response to Reviewer's comments	3
Introduction	3
Body	3
Other Results	8
Key Research Accomplishment	9
Reportable Outcomes	9
Conclusions	10
Literature Cited	10
Presentations	11
Appendices	Submitted with the earlier version

Response to Reviewer's Comments:

This is the revised report for the grant number W81XWH-04-C-0071. The main suggestion of the reviewer was to format the progress report to address each task separately. Because, in addition to the work specified in the tasks, we also studied questions that arose during the study, we have presented separate sections for all observations (IIA) and task-related progress (IIB) in the body of the report. This also led to some changes in the References section. At present, the attachments remain the same (i.e., those that were submitted with the earlier version of the report). When the submitted papers are published, we will send PDF copies to USAMRMC.

I. INTRODUCTION

The central nervous system (CNS) and the immune system communicate bidirectionally, and cholinergic agents modulate the immune system. Organophosphates, such as the nerve gas sarin, are powerful irreversible inhibitors of ChE, leading to neurotoxicity, seizures, and death. Because of the ease and low cost of production, sarin gas is a tool of mass destruction in the hands of terrorist groups and rogue nations. While people in the immediate vicinity of sarin attack may receive neurotoxic doses, people away from this area are likely to receive sublethal exposures. Even subclinical doses of sarin cause subtle changes in the brain, and subclinical exposure to sarin have been proposed as an etiology to the Gulf War Syndrome. Our experiments suggested that low doses of sarin suppress the immune system and glucocorticoid production (Kalra et al., 2002), but stimulate the production of proinflammatory cytokines in the brain (Henderson et al., 2002). Moreover, sarin exposures have been shown to cause lung inflammation (Pant et al., 1993). Therefore, it appears that while sarin exposure may inhibit adaptive immunity, it might stimulate inflammatory responses. Moreover, sarin may affect the hypothalamus-pituitary-adrenal (HPA) axis, thereby suppressing glucocorticoid (corticosterone) production and aggravating an inflammatory response. However, the mechanism by which sarin affects the adaptive and innate (inflammatory) immune responses is not clear, and we proposed the following specific aims:

Task 1: Determine whether cholinergic agents require access to the CNS to alter the immune response and corticosterone levels

Task 2: Determine the steps in the antigen-induced T cell signaling pathway that are affected by low-dose sarin inhalation.

Task 3: Ascertain the role of the sympathetic autonomic nervous system in sarin-induced immunotoxicity.

Task 4: Ascertain the role of muscarinic and nicotinic acetylcholine receptors play in sarin-induced immunotoxicity.

II. BODY

In additions to the specific aims proposed in the grant application, as the studies progressed, we were able to address emerging questions that were not covered under the stated tasks. To provide some cohesiveness to the results, we will first present the results in the sequence as they developed (IIA). At the end of this section, we will present the studies and the results as they relate to the tasks proposed in the application (IIB).

IIA: Results Obtained During the Funding Period

1. **Role of the Blood-brain-barrier (BBB) in cholinergic immunotoxicity.** We have previously shown that the ganglionic blocker chlorisondamine (CHS) attenuated sarin-induced immunosuppression (Kalra et al., 2002), indicating that sarin affected the immune system through the autonomic nervous system. To understand the neuroimmune effects of sarin and how cholinergic compounds affect the immune response, we determined whether the cholinergic compounds needed to reach the central nervous system (CNS) to induce immunosuppression. Therefore, we compared the immunological effects of cholinergic compounds that crossed the BBB with those that did not cross the BBB. Our results clearly demonstrated that the cholinergic compounds that cross BBB, inhibit the immune response when given by inhalation or intracerebroventricularly (ICV), however, poorly BBB permeable cholinergic compounds, unless given in very high doses, are immunosuppressive only when administered directly (intracerebroventricularly) into the brain. These results also suggested that subcutaneous administration of pyridostigmine bromide at doses equivalent to those given to the Gulf War veterans had no significant effect on any of the immunological parameters tested, unless the drug was given directly into the brain. These observations were published in Langley et al., 2004a (**Appendix 1**). ; *Please note: While the preliminary data on the effects of pyridostigmine bromide on the immune system were performed with money from the acknowledged NIH grants in the paper, most of the data reported in Langley et al. paper were performed with the funds from this grant.*
2. **Long- and short-term changes in the neuroimmune endocrine function after sarin inhalation.**

Our previous studies (Kalra et al., 2002) indicated that subclinical doses of sarin induced changes in the neuroimmune and endocrine functions. To ascertain the mechanism and the life of these changes, we examined several immunologic/inflammatory parameters in sarin-exposed rats. These observations were published in Pena-Philippides et al. 2007 (**Appendix 2**) and summarized below:

 - (a) **Sarin induces the expression of proinflammatory cytokines in the lung**

Because acute exposure to high doses of sarin (1 Lct₅₀) caused serious lung inflammation in rats (Pant et al., 1993), we examined the expression of the proinflammatory cytokines IL-1 β , TNF- α and IL-6 by RT-PCR in the rat lung after single inhalation of a subclinical dose of sarin (0.4 mg/m³ for 1 h). It was observed that sarin increases the lung expression of proinflammatory cytokines within 24 h after sarin exposure. This provides a molecular basis for the lung inflammation seen in animals exposed to high doses (\geq LCT₅₀) of sarin (Pant et al., 1993).
 - (b) **Sarin stimulates the nuclear translocation of NF κ B**

The transcription factor NF κ B plays a critical role in the expression of proinflammatory cytokines such as TNF- α , IL-1 β , and IL-6, and requires the migration of NF κ B from the cytoplasm to the nucleus. Because sarin stimulated the expression of these cytokines in the lung, we ascertain whether the effect was mediated through activation of NF κ B. Our results (Pena-Philippides et al., 2007; Appendix 2), shows that sarin strongly stimulates the nuclear translocation of NF κ B in bronchoalveolar lavage cells from sarin-treated animals. Thus, increased lung expression of proinflammatory cytokines in sarin-treated animals might result from the activation of NF κ B.
 - (c) **Effects of sarin on innate and adaptive immunities are short-lived, and the effects of sarin on the adaptive immunity are mediated through the central nervous system**

Because two of the Japanese sarin terrorism survivors succumbed to Legionella infection nearly two years after the sarin exposure (Kamimura et al., 1998), there was a concern that sarin might have long-term immunological effects that would weaken the resistance to relatively ubiquitous lung organism, Legionella. However, our results indicated that both adaptive (antibody and T cell receptor-mediated responses) as well inflammatory responses were relatively short-lived (i.e., undetected 2-4 weeks after sarin exposure). Moreover, the immunosuppressive effects of sarin on the antibody response were blocked by pretreatment with the ganglionic blocker chlorisondamine, suggesting the immunological effects of sarin might be mediated through the autonomic nervous system.

(d) Sarin affects the hypothalamus-pituitary adrenal (HPA) axis

We have previously shown that subclinical doses of sarin decrease the serum levels of corticosterone in rats. To ascertain the life-span of this effect and whether sarin affected the glucocorticoid level through the HPA axis or selectivity through adrenal toxicity, we determined the changes in the ACTH in parallel with serum corticosterone (CORT) levels over a period of several weeks after sarin exposure. Our results suggested that in addition to CORT, sarin also suppressed ACTH production. Moreover, the changes in CORT/ACTH were detectable even at 8 weeks after sarin exposure. These results suggest that sarin has relatively long-lasting effects on the HPA axis.

3. **Sarin-induced respiratory failure is mainly contributed by the central chemoreceptor dysfunction**

It is well established that most mortality associated with sarin exposure results from respiratory failure (Sidell and Borak, 1992; Sidell, 1994). While the mechanism of the respiratory failure is not known, it is generally believed that very high levels of acetylcholine released at the nerve termini after high-dose sarin exposure causes bronchoconstriction. However, our experiments indicate that while sarin does cause bronchoconstriction, it is not the main reason for the respiratory failure. It appears that sarin causes ventilatory crisis that emanates from increased hypercapnia, decreased hypoxia, and decreased blood pH. Moreover, the diaphragm fails to respond to hypercapnia, which is primarily associated with the failure of the central respiratory chemoreceptors to sense hypercapnia and hypoxia. These results are presented in Zhuang et al. (Manuscript in preparation; a rough draft of the manuscript is attached as **Appendix 3**). *Please note: we are still working on this paper and some of the sections are incomplete. We believe that the paper would be ready for submission to the Am. J. Respir. Critical Care Med. within next 2-4 weeks.* Some of the major findings presented in this paper are summarized below:

(a) Sarin affects arterial blood gases and pH

We observed strong ventilatory changes in animals exposed to high doses of sarin inhalation, leading to respiratory failure and death in number of animals. To determine whether the ventilatory changes in sarin-exposed animals reflected changes in P_{CO_2} , we measured the arterial blood P_{CO_2} , P_{O_2} , and pH, and observed that rats exposed to LCt₅₀ sarin has significantly higher P_{CO_2} , lower P_{O_2} , and lower arterial blood pH. Thus, under the baseline conditions, sarin causes hypoxia and hypercapnia, which leads to acidosis (decreased blood pH).

(b) Sarin markedly depresses ventilatory responses to hypercapnia and hypoxia

Normal animals increase respiratory frequency when exposed to hypercapnia; however, animals exposed to high doses of sarin failed to increase respiratory frequency after exposure to 7% CO₂. In fact, the frequency response to hypercapnia was essentially eliminated in animals exposed to LCt₅₀ sarin.

- (c) Sarin impairs chemosensory function of the central respiratory chemoreceptors
To ascertain whether the ventilatory changes associated with high-dose sarin exposure resulted from sarin-induced paralysis of the diaphragm muscle and/or effects on the central respiratory chemoreceptors, electromyogram of the diaphragm was recorded before and after the activation of the phrenic nerve. Prior to electric stimulation of the nerve, the spontaneous breathing activity of sarin-treated animals was barely discernable (flat signal); however, the contractions of the diaphragm muscle resumed after electric stimulation of the phrenic nerve. These results strongly suggest that the respiratory failure associated with the LCt₅₀ dose of sarin results primarily from the effects of sarin on the central respiratory chemoreceptors, which fail to sense hypercapnia.

4. **Acute and chronic silicosis differ mechanistically**

Although this was not part of the original tasks specified in this project, however, several years back we had received a grant (pilot project) from the Army to study immunological effects of silica inhalation. In that project we discovered a biphasic response to inhalation of occupationally relevant doses of silica. The observations were published in Langley et al. (2004b). We also recognized that the commonly used animal models for human silicosis modeled only the rare form of human silicosis (i.e., acute silicosis) and not the most prevalent form of silicosis (chronic silicosis). There was some indication that the two forms of silicosis may arise through different mechanisms. Because silica exposure might be a factor in the Gulf War Syndrome, we decided to delineate the mechanism of acute and chronic silicosis, and found that while acute silicosis arises by severe lung injury and apoptosis, chronic silicosis produces limited inflammation and a strong anti-apoptotic response. These results have been recently submitted for publication to the Am. J. Respir. Cell Mol Biol. (see Langley et al., **Appendix 4**).

IIB: Progress Report as it relates to specific tasks

Task 1: Determine whether cholinergic agents require access to the CNS to alter the immune response and corticosterone levels.

Approach and results: Although both sarin and pyridostigmine bromide inhibit acetylcholine esterase activity; our results indicated that while sarin suppressed various parameters of adaptive immunity, pyridostigmine bromide had no discernible effect on these parameters. Because, unlike sarin, pyridostigmine bromide does not normally cross the blood-brain-barrier (BBB), we hypothesized that the immunologic effects of cholinergic agents may depend on their ability to cross the BBB. This hypothesis was borne by our experiments, where we demonstrated that while BBB-permeable compounds such as sarin and physostigmine suppressed T-cell functions, the BBB-impermeable cholinergic compounds such as pyridostigmine bromide and edrophonium did not significantly affect these responses. This was further confirmed by the observation that intracerebroventricular administration of pyridostigmine and edrophonium, even at relatively low concentrations, suppressed T cell responses. These results were published in Langley et al., 2004a (**Appendix 1**). Moreover, the results supported our earlier findings (Kalra et al., 2002) that the immune effects of sarin are mediated by the autonomic nervous system.

Task 2: Determine the steps in the antigen-induced T cell signaling pathway that are affected by low-dose sarin inhalation.

Approach and results: We previously reported that chronic exposure to subclinical doses of sarin, significantly suppresses T cell mitogenesis (Kalra et al., 2002). To determine whether these changes are long-lived, we examined the T cell responses (Con A- and anti-T cell receptor antibody-mediated T cell proliferation) after exposure to sarin. As described in section 2C of the Body, most effects on T cell proliferation waned within 4 weeks after sarin exposure. Moreover, changes in the anti-TCR induced $[Ca^{2+}]_i$ were also normalized during this period (Pena-Philippides et al., 2007 – **Appendix 2**).

During work on this project, we learnt that exposure of guinea pigs to high doses of sarin via inhalation caused lung inflammation (Levy et al., 2004), which we thought might be related to the respiratory effects of sarin observed in humans. This also suggested that in addition to T cells, sarin may also affect the function of other immune cells. Therefore, we exposed rats to subclinical doses of sarin and determined its effects on lung inflammation. We did not observe significant migration of leukocytes in the lung; however, the bronchoalveolar lavage cells clearly displayed the molecular imprints of lung inflammation. These include increased nuclear translocation of the proinflammatory transcription factor, NF κ B in the BAL cells, and in the lung tissue increased transcription of proinflammatory cytokines TNF- α , IL-1 β , and IL-6 (**Appendix 2**), and increased mRNA and protein for substance P (unpublished observation). These results suggest that at least one of the effects of sarin may be to affect the MAP kinase pathway, leading to the activation of NF κ B. However, whether sarin affects the MAP kinase pathway in T cells has not been examined as yet. We have some preliminary evidence to suggest that sarin may impair the activation of protein tyrosine kinases following the ligation of the T cell receptor with anti-TCR antibodies to simulate antigen stimulation (Razani-Boroujerdi et al., unpublished observation). We hope we will be able to explore this area further in our new grant.

Task 3: Ascertain the role of the sympathetic autonomic nervous system in sarin-induced immunotoxicity.

Approach and results: Prior to the activation of this project, we had preliminary evidence to suggest that some of the neuroimmune endocrine effects of sarin might be mediated through the autonomic nervous system (Kalra et al., 2002). We have previously shown that subclinical doses of sarin markedly decrease serum glucocorticoid levels (Kalra et al., 2002). To ascertain whether the effects are regulated by the autonomic nervous system, we pretreated animals with the ganglionic blocker chlorisondamine and exposed them to various cholinergic agents including sarin, physostigmine, edrophonium, and pyridostigmine bromide. The results indicated that in each case the cholinergic agent decreased serum corticosterone level that was abrogated by pretreatment with chlorisondamine (Langley et al., 2004; Appendix 1). Thus, the autonomic nervous system plays an important role in the regulation of glucocorticoid levels in the sarin-exposed animals.

Changes in the autonomic nervous system affect the function of the hypothalamus-pituitary-adrenal axis and, in order to ascertain, whether sarin-induced suppressive effects on serum corticosterone levels reflected changes in the HPA axis, we determined the plasma adrenocorticotrophin hormone (ACTH) level in control and sarin-treated animals. Indeed, the changes in corticosterone paralleled those of ACTH, indicating that sarin affects the HPA axis. Given that chlorisondamine blocks the effects of sarin on serum glucocorticoid levels, suggests that the effects are regulated by the autonomic nervous system.

Task 4: Ascertain the role of muscarinic and nicotinic acetylcholine receptors play in sarin-induced immunotoxicity

Approach and results: We have demonstrated that nicotinic and acetylcholine receptors on immune cells modulate their immune/inflammatory responses, and activation of nicotinic acetylcholine receptors leads to immunosuppression (Razani-Boroujerdi et al., 2008). Moreover, the main nicotinic acetylcholine receptor (nAChR) type on T cells is $\alpha 7$ -nAChR (Razani-Boroujerdi et al., 2007). We surmised that the immunosuppressive activity of sarin might be related to activation of $\alpha 7$ -nAChRs and, therefore, $\alpha 7$ -nAChR knockout animals may not respond to the immunosuppressive effects of sarin. Unfortunately, $\alpha 7$ -nAChR KO mice are sickly and extremely expensive (\$360 + special airfare/animal). Nonetheless, because of the potential results, we purchased 12 WT and 12 $\alpha 7$ -nAChR-KO mice from the Jackson laboratory and grouped them into two sub-groups of 6 animals/group and exposed them to vehicle or sarin. While the anti-SRBC response of sarin-treated WT animals was significantly lower with tight standard deviation, the antibody response of KO mice was extremely variable, and we were unable to derive any firm conclusions from the results and, therefore, did not include them in the earlier progress report. Two of the 6 animals showed somewhat lower SRBC response, but the group as a whole did not show any significant difference. These experiments need to be repeated with much larger n value and, hopefully, we would be able to do these experiments under new grant.

Other results (not as yet put together for publication)

Some of the other results from our studies that have not been yet put into a manuscript form are summarized below:

- (a) Sarin increases the expression of Substance P (SP)
Sarin affects a number of inflammatory parameters in the lung. SP is known to promote lung inflammation through production of proinflammatory cytokines via NF κ B activation (Okaya et al., 2004; Azzolina et al., 2003), and sarin activates NF κ B (Appendix 2). Moreover, organophosphate pesticides induce SP. Therefore, we determined whether sarin affects SP transcription in the lung. Indeed, a single subclinical exposure to sarin significantly increased the transcription of SP at 24 h after sarin exposure. Preliminary experiments also suggest that immunoreactive SP is increased in the bronchoalveolar lavage by approximately 3-fold. Thus, sarin stimulates SP expression in the lung, and the increased production of SP might contribute to sarin-induced NF κ B activation and lung inflammation.
- (b) Sarin increases airway resistance in response to methacholine
Bronchoconstriction is one of the serious consequences of exposure to high-dose sarin. High-dose exposure to organophosphate pesticides not only induces bronchoconstriction but increases the density of muscarinic receptors in the lung, making them more sensitive to muscarinic agonist. To ascertain whether exposure to sarin increases the airway resistance response to muscarinic agonists, rats were exposed to a single dose of 0.5 LC₅₀ sarin. The airway resistance of these animals was determined by plethysmography immediately following sarin exposure. Results indicated that sarin significantly increases the airway resistance of the lungs to methacholine.
- (c) Sarin increases the mRNA expression of muscarinic and nicotinic receptors in the lung
To determine whether the increased airway resistance to methacholine was contributed by increased expression of muscarinic acetylcholine receptors (mAChRs) in the lung in response to sarin, animals were exposed to 0.5 LC₅₀ sarin and, after 24 h, the lung

mRNA was analyzed for the expression of M1, M2, and M3 mAChRs by RT-PCR. The results indicated that sarin increased the expression of M1 and M3, but not M2 mAChRs in the lung. This could explain the increased methacholine sensitivity of sarin-treated lungs. Interestingly, however, the sarin treatment also significantly elevated the expression of $\alpha 7$ -nicotinic acetylcholine receptors in the lung. At this time, we have not figured out the significance of the increased expression of the nicotinic receptors in sarin-treated animals.

- (d) High-dose sarin induces neuronal death in hippocampus and cortex
Increasing evidence suggested that the survivors of the Japanese sarin attack are more likely to develop cognitive and memory impairment (Yanagisawa et al., 2006 – 16962140; Suzuki, 2007). Our recent results suggest that surviving rats at 24 h after LCt₅₀ sarin exposure exhibit neuronal death in several brain areas particularly the CA4 region of the hippocampus and piliform cortex. Therefore, it is likely that these animals might develop cognitive impairment in the long run, and would serve as a model for the long-term neurotoxicity/memory impairment seen in humans exposed to high-dose sarin.

KEY RESEARCH ACCOMPLISHMENTS

- Exposure to subclinical doses of sarin suppresses the immune system and CORT production, and the effects are at least partially ameliorated by pretreatment with ganglionic blockers. Moreover, the changes in serum CORT levels are paralleled by the changes in the plasma ACTH levels, indicating that sarin affects the HPA axis.
- Cholinergic agents that cross the blood-brain-barrier cause immunotoxicity similar to sarin.
- Most immunological/inflammatory effects of sarin are temporary (i.e., lasting for 1-4 wk) after sarin exposure; however, the suppression of serum CORT levels remain detectable at least until 8 weeks after sarin exposure.
- Sarin increases the expression of muscarinic and nicotinic acetylcholine receptors, and airway resistance in the lung. It also upregulates the markers of neurogenic inflammation in the lung.
- Sarin increases MAP kinase (e.g. ERK) and NF κ B activities in BAL cells; changes in the ERK and NF κ B could explain the increase in the inflammatory response and production of proinflammatory cytokines.
- Immediate mortality associated with inhalation of high-dose sarin is related to respiratory failure. The latter results primarily from the failure of the central respiratory centers to respond to hypercapnia/hypoxia.
- At LCt₅₀, sarin damages central nervous system, particularly the CA4 region of the hippocampus and some areas of the cortex. These injuries to the central nervous system may produce long-term neurobehavioral changes akin to those seen in the survivors of sarin terrorism in Japan (...).

REPORTABLE OUTCOMES

1. Raymond J. Langley, Roma Kalra, Neerad Mishra, Mohan L. Sopor. 2004. Central but not the peripheral action of cholinergic compounds suppress the immune system. *J. Neuroimmunol.* 148: 140-145. (**Appendix 1**).
2. Pena-Philippides, J. C., Seddigheh Razani-Boroujerdi, Raymond J. Langley, Neerad C. Mishra, Shashi P. Singh, Rogene F. Henderson, and Mohan L. Sopor. 2007. Long- and short-term changes in the neuroimmune-endocrine parameters following exposures of F344 rats to low-dose sarin inhalation. *Toxicol. Sci.* 97: 181-188. (**Appendix 2**).

3. J. Zhuang, F. Xu, M. Campen, C. Zhang, J. C. Pena-Philippides, and M. L. Soporì. Acute sarin exposure attenuates ventilatory responses to hypercapnia and hypoxia in conscious Rats. (*Submitted to Toxicol. Appl. Pharmacol.*). (**Appendix 3**).
4. Raymond J. Langley, Neerad C. Mishra, Juan Carlos Peña-Philippides, Seddigheh Razani-Boroujerdi, Julie A. Hutt, and Mohan L. Soporì. Biphasic response to silica. II. Acute and chronic silicosis differ in apoptotic response. (*Submitted to Am. J. Respir. Cell Mol. Biol.*). (**Appendix 4**).
5. Seddigheh Razani-Boroujerdi, Juan Carlos Peña-Philippides, Mathew Campen, Neerad Mishra, Shashi Singh, and Mohan Soporì. Sarin causes bronchoconstriction and neurogenic inflammation in the lung and upregulates the expression of muscarinic and nicotinic acetylcholine receptors. (*Manuscript to be prepared*).

CONCLUSIONS

Subclinical exposure to cholinergic agents, such as sarin, pesticides, and other organophosphates suppress the immune system, and this immunotoxicity is dependent on their ability to cross the BBB. The effects are mediated through the autonomic nervous system and are at least partially overcome by ganglionic blockers. Sarin and other cholinergic agents inhibit glucocorticoid production and, for sarin, the effect may result through suppression of the HPA axis. Changes in CORT and ACTH levels may represent biomarkers of cholinergic toxicity. The increased expression of proinflammatory cytokines in the brain of sarin treated animals might damage the central respiratory chemoreceptors and hippocampal neurons, leading to respiratory failure and long-term cognitive deficits, respectively. Lung inflammation in high-dose sarin-exposed animals may result from neurogenic inflammation and the production of proinflammatory cytokines in the lung. Ganglionic blockers may have some therapeutic value in ameliorating the immunosuppressive and inflammatory effects of sarin.

LITERATURE CITED

- Azzolina, A., A. Bongiovanni, and N. Lampiasi. 2003. Substance P induces TNF-alpha and IL-6 production through NF kappa B in peritoneal mast cells. *Biochim. Biophys. Acta* 1643: 75-83.
- Henderson, R. F., E. B. Barr, W. B. Blackwell, C. R. Clark, C. A. Conn, R. Kalra, T. H. March, M. L. Soporì, Y. Tesfaigzi, M. G. Ménache, and D. C. Mash. 2002. Response of rats to low levels of sarin. *Toxicol. Appl. Pharmacol.* 184: 67-76.
- Kalra, R., S. P. Singh, S. Razani-Boroujerdi, R. J. Langley, W. B. Blackwell, R. F. Henderson, and M. L. Soporì. 2002. Subclinical doses of the nerve gas sarin impair T cell responses through the autonomic nervous system. *Toxicol. Appl. Pharmacol.* 184: 82-87.
- Kamimura, M., O. Katoh, H. Kawata, K. Kudo, Y. Yagishita, H. Niino, K. Saitoh, and A. Saitoh. 1998. Legionella pneumonia caused by aspiration of hot spring water after sarin exposure. *Nihon. Kokyuki. Gakkai. Zasshi.* 36:278-82.
- Langley, R.J., R. Kalra, N. Mishra, and M. L. Soporì. 2004. Central but not the peripheral action of cholinergic compounds suppress the immune system. *J. Neuroimmunol.* 148:140-145.
- Langley, R. J., R. Kalra, N. C. Mishra, F. F. Hahn, S. Razani-Boroujerdi, S. P. Singh, J. M. Benson, J. C. Pena-Philippides, E. B. Barr, and M. L. Soporì. 2004b. A biphasic response to silica: I. Immunostimulation is restricted to the early stage of silicosis in lewis rats. *Am. J. Respir. Cell Mol. Biol.* 30: 823-829.
- Levy A, Chapman S, Cohen G, Raveh L, Rabinovitz I, Manistersky E, Kapon Y, Allon N, Gilat E. 2004. Protection and inflammatory markers following exposure of guinea pigs to sarin vapour: comparative efficacy of three oximes. *J. Appl. Toxicol.* 24: 501-504.

- Okaya, T., R. Holthaus, A. Kato, and A. B. Lentsch. 2004. Involvement of the neuropeptide substance P in lung inflammation induced by hepatic ischemia/reperfusion. *Inflamm. Res.* 53: 257-261.
- Pant, S. C., R. Vijayaraghavan, and S. Das Gupta. 1993. Sarin induced lung pathology and protection by standard therapy regime. *Biomed. Environ. Sci.* 6: 103-111.
- Razani-Boroujerdi, S., M. Behl, F. F. Hahn, J. C. Pena-Philippides, J. Hutt, and M. L. Sopori. 2008. Role of muscarinic receptors in the regulation of immune and inflammatory responses. *J. Neuroimmunol.* 194:83-88.
- Razani-Boroujerdi, S., R. T. Boyd, M. I. Davila-Garcia, J. S. Nandi, N. C. Mishra, S. P. Singh, J.C. Philippides, R. Langley and M. L. Sopori: 2007. T cells express $\alpha 7$ -nicotinic acetylcholine receptor subunits that require a functional TCR and leukocyte-specific tyrosine kinase for nicotine-induced Ca^{2+} response. *J. Immunol.* 179: 2889-2898.
- Sidell, F.R. 1994. Clinical effects of organophosphorus cholinesterase inhibitors. *J Appl Toxicol.* 14:111-3.
- Sidell, F.R., and J. Borak. 1992. Chemical warfare agents: II. Nerve agents. *Ann. Emerg. Med.* 21:865-71.
- Slattery DA, Neumann ID. 2008. No stress please! Mechanisms of stress hyporesponsiveness of the maternal brain. *J Physiol* 386: 377-385.
- Yanagisawa, N, H. Morita, and T. Nakajima. 2006. Sarin experiences in Japan: acute toxicity and long-term effects. *J. Neurol. Sci.* 249:76-85.

PRESENTATIONS

Invited Lectures given by the PI (Mohan Sopori) related to sarin studies

- | | |
|-----------------|--|
| May 17, 2004 | Neuroimmune Effects of Sarin and Other Cholinergic Agents Are Mediated Through the Autonomic Nervous System: A Possible Role for IL-1 β in this Response. The Medical Defense Biosciences Review (US Army Medical Defense). Hunt Valley, MD. |
| March 7, 2005: | Immunotoxicity of inhaling sarin and other cholinergic agents. 44 th Annual meeting of Society of Toxicology meeting, New Orleans, LA. |
| April 6, 2006 | Role of muscarinic and nicotinic receptors in neuroimmune modulation. 12 th Conference of the Society on Neuroimmune Pharmacology (SNIP), Santa Fe, NM. |
| May 2, 2006 | Sarin-induced neuroimmune modulation and lung injury. Military Health Research Forum, May 2, 2006, San Juan, PR |
| August 14, 2006 | Alterations in cholinergic receptors, cytokines, glucocorticoids, and immunity following low-dose cholinergic exposure. Meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses, VA Headquarters, Washington, DC. |
| August 9, 2007 | Respiratory and neuroimmune effects of sarin inhalation. 10 th Annual Force Health Protection Conference; Louisville, Ky. |

Upcoming Invited Lectures:

- | | |
|-----------------|--|
| August 11, 2008 | Neuroimmune effects of sarin inhalation. 11th Annual Force Health Protection (FHP) Conference, 8 - 15 August 2008 in |
|-----------------|--|

Albuquerque, NM. Hosted by The U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM).

August 12, 2008

Mechanism of silicosis. 11th Annual Force Health Protection (FHP) Conference, 8 - 15 August 2008 in Albuquerque, NM. Hosted by The U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM).

APPENDICES

Appendices were submitted with the previous version of the progress report.